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ARTICLE

Association between mannose-binding lectin, high-sensitivity C-reactive protein and the progression of diabetic nephropathy in type 1 diabetes

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Abstract
Aims/hypothesis Diabetic nephropathy has been associated with low-grade inflammation and activation of the complement system in cross-sectional studies. Data from prospective studies are sparse. We investigated the associations of the complement activator mannose-binding lectin (MBL) and the inflammatory marker high-sensitivity C-reactive protein (hsCRP) with the development of nephropathy in a large prospective study of patients with type 1 diabetes from the Finnish Diabetic Nephropathy (FinnDiane) Study.
Methods Baseline MBL and hsCRP were measured in 1,564 type 1 diabetes patients from the FinnDiane study, of whom 1,010 had a normal albumin excretion rate, 236 had microalbuminuria and 318 had macroalbuminuria. The main outcome was progression in renal disease during follow-up.
Results Both baseline MBL ($p=0.038$) and hsCRP ($p<0.001$) increased with increasing level of albuminuria. During 5.8± 2.2 years of follow-up, progression to a higher albuminuria level or end-stage renal disease (ESRD) occurred in 201 patients. MBL levels were higher in progressors compared with non-progressors at all steps of progression, and in a covariate adjusted multivariate Cox-regression analysis MBL levels above the median were significantly associated with progression from macroalbuminuria to ESRD (hazard ratio 1.88, 95% CI 1.06–3.32, $p=0.030$). In a univariate analysis, hsCRP levels above the median were significantly associated with progression from normal albumin excretion rate to microalbuminuria, but the association was only borderline significant after adjustment for covariates (hazard ratio 1.56, 95% CI 0.97–2.51, $p=0.068$).
Conclusions/interpretation This study demonstrates that concentrations of both MBL and hsCRP are associated with the progression of renal disease in type 1 diabetes.

Keywords Complement system · Complications · Diabetes · Immune system · Mannose-binding lectin · Nephropathy

Abbreviations

AER	Albumin excretion rate
ESRD	End-stage renal disease
FinnDiane	Finnish Diabetic Nephropathy Study
hsCRP	High-sensitivity C-reactive protein
MBL	Mannose-binding lectin
HR	Hazard ratio

T. K. Hansen and C. Forsblom contributed equally to this study.

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Introduction

Despite significant improvements in diabetes care with intensified treatment of hyperglycaemia and hypertension, a significant proportion of patients with type 1 diabetes still develop diabetic nephropathy [1]. It is generally believed that the pathogenesis of diabetic renal disease is multifactorial, and progression may involve both low-grade inflammation and activation of the complement system.

The complement system is an enzyme cascade of the innate immune system that is activated via one of three different pathways: the classical, the alternative and the mannose-binding lectin (MBL) pathways [2, 3]. The system has evolved as a defence against pathogens, but it may act as a double-edged sword and cause self-damage following adverse activation. This happens through deposition of the membrane attack complex (MAC, also called C5b-9), the cytolytic endproduct of the complement cascade, or through increased inflammation via release of the anaphylatoxins C5a and C3a. Increased deposition of MAC is found in diabetic kidneys [4], and recent experimental data indicate that glycoxidation-mediated local complement activation may be a significant contributor to the progression of diabetic nephropathy [5]. This finding thus provides a possible link between hyperglycaemia and progression of diabetic kidney disease.

Animal studies have shown that *MBL2* knockout mice that are unable to activate the MBL pathway of the complement system are protected from the detrimental effects of diabetes on the kidneys [6], indicating that the MBL pathway plays a significant role in diabetic renal changes. In humans, MBL levels vary significantly from person to person because of frequently occurring polymorphisms within exon 1 as well as in the promoter region of the *MBL2* gene on chromosome 10 [7, 8]. The *MBL2* gene is not a type 1 diabetes susceptibility gene per se [9], but patients with type 1 diabetes have significantly increased serum levels of MBL [10, 11], and both high circulating levels of MBL and high-coding MBL genotypes are associated with the presence and development of microalbuminuria and diabetic nephropathy [12–14].

There is evidence of increased inflammatory activity in type 1 diabetes [15, 16]. Markers of inflammation such as high sensitivity C-reactive protein (hsCRP) have been cross-sectionally associated with diabetic nephropathy in some studies [17], while in the DCCT/Epidemiology Of Diabetes Interventions And Complications (EDIC) study CRP levels were not associated with complications [16]. The relationship between CRP levels and glycaemic control is complex, and CRP levels may even increase with intensified treatment depending on the degree of weight gain associated with the treatment [18]. Circulating hsCRP levels may predict the development of albuminuria in longitudinal studies of patients with type 2 diabetes [19, 20]. Whether this is also the case in type 1 diabetes remains unclear, although there was no clear association between CRP level and the progression of nephropathy in the DCCT trial [21]. Circulating levels of MBL in patients with type 2 diabetes are not different from those of healthy people, but MBL alone and especially in combination with hsCRP provides prognostic information on the development of albuminuria during the long-term follow-up of normoalbuminuric patients with type 2 diabetes [22]. In the present study we investigated the association of the prevalence and incidence of diabetic nephropathy with MBL and hsCRP levels at baseline in a large prospective study of patients with type 1 diabetes from the Finnish Diabetic Nephropathy Study (FinnDiane).

Methods

Study design This study was part of the ongoing prospective FinnDiane Study, which is a nationwide, comprehensive multicentre study with the aim of identifying genetic and environmental risk factors for diabetic nephropathy in type 1 diabetes patients.

At baseline, patients underwent a thorough clinical investigation at a regular visit to the attending physician. The prospective study started in 2005. For all patients in the present analysis, all available medical files, including laboratory data, were reviewed and changes in renal status were verified.

Study participants Out of an original cohort of 1,691 patients with data available on both serum CRP and MBL, 127 with CRP above 10 mg/l were excluded because of potential ongoing infection. Therefore, 1,564 patients participated in the present study and were followed for an average of 5.8±2.2 years. Type 1 diabetes was defined as onset of diabetes before the age of 35 years and permanent insulin treatment initiated within 1 year of diagnosis.

The cohort was subdivided into 1010 patients with normal urinary albumin excretion rate (normoalbuminuria; albumin excretion rate [AER] <20 μg/min or <30 mg/24 h), 236 patients with microalbuminuria (20≤AER ≤200 μg/min or 30<AER < 300 mg/24 h) and 318 patients with macroalbuminuria (AER≥200 μg/min or ≥ 300 mg/24 h), based on fulfilment of the criteria for AER in at least two out of three consecutive overnight or 24 h urine collections at baseline. All urine samples were screened with a urine stick to exclude any urinary tract infection. Patients with end-stage renal disease (ESRD), defined as patients on dialysis or having received a kidney transplant, were excluded.

Progression of renal disease was defined as follows. All local AER data between baseline and the follow-up visit

were reviewed. Based on the AER in any two out of three consecutive urine collections during the follow-up period, the renal status of patients was classified as in the baseline examination. Progression was defined as a change from one level to a higher level of albuminuria or the development of ESRD. Patients without progression of renal disease and patients who regressed to a lower level of albuminuria were classified as non-progressors.

The ethics committees of all participating centres approved the study protocol. Written informed consent was obtained from each patient and the study was performed in accordance with the Declaration of Helsinki as revised in 2000.

Measurements At the regular patient visits, data on medication and diabetic complications were recorded using a standardised questionnaire, which was completed by the patient's attending physician based on medical files. Blood pressure was measured twice in the sitting position with a mercury sphygmomanometer after a 10 min rest and the average of these measurements was used in the analysis. Height, weight and WHR were recorded, and blood was drawn for the measurement of HbA_{1c}, lipids, creatinine, hsCRP and MBL.

HbA_{1c} was determined by standardised assays at each centre. Serum lipid and lipoprotein concentrations were measured by automated enzymatic methods using a Cobas Mira analyser (Hoffmann-La Roche, Basle, Switzerland). Serum creatinine was assessed by enzymatic methods at a central laboratory. AER was determined in one 24 h urine collection at the central laboratory by radioimmunoassay (Pharmacia, Uppsala, Sweden) or immunoturbidimetry. The serum concentration of MBL was determined using an in-house time-resolved immunofluorometric assay [10], and hsCRP was measured by a photometric, immunochemical method with an ultrasensitive kit (Orion Diagnostica, Espoo, Finland). The within- and between-assay CVs were 4.0% and 6.5%, respectively.

Statistical analysis Data are expressed as mean ± SD for normally distributed values and as median with interquartile range for non-normally distributed values. Differences between groups for normally distributed variables were tested using ANOVA and non-parametric data with the Kruskal–Wallis test. Categorical variables were analysed with the χ^2 test. Risk factors for the progression of diabetic nephropathy were assessed using Cox regression analysis. In the normoalbuminuric group, with the observed number of progressors, the study had an 80% power with an alpha level of 0.05 to detect a difference in CRP or MBL of 0.33 SDs. The corresponding differences for patients with microalbuminuria and macroalbuminuria at baseline were 0.52 and 0.36 SD. All calculations were performed with SPSS 15.0.1 (SPSS, Chicago, IL, USA).

Results

Baseline values Clinical characteristics of the patients grouped by albuminuria status are presented in Table 1. There was a stepwise increase in median serum MBL levels with increasing albuminuria level (overall $p=0.038$), and patients with macroalbuminuria had significantly higher levels of MBL than patients with normoalbuminuria. Concentrations of hsCRP were also increased in patients with albuminuria at baseline (overall $p<0.001$), but MBL and hsCRP levels did not correlate ($r=0.015$, $p=0.56$). Median MBL levels were slightly higher in men (1864 μg/l, IQR 634-3100) than in women (1,643 μg/l, IQR 505–2,947; $p=0.029$), while hsCRP concentrations were significantly higher among women (2.10 mg/l, IQR 1.41–3.62) than among men (1.72 mg/l, IQR 1.07–3.09, $p<0.001$). The frequency of use of non-steroidal anti-inflammatory agents (NSAIDs) was 3.9%, 6.0% and 15.3%, and the use of statins 3.5%, 7.9% and 21.8% in patients with normal AER, microalbuminuria and macroalbuminuria, respectively. When comparing the CRP and MBL concentrations within the three AER categories separately, there were no significant differences between those with and without NSAID or statin treatment.

Progression The mean follow-up time was 5.8±2.2 years, and during this period progression to either the next albuminuria level or ESRD occurred in 201 patients (Table 2). Baseline serum MBL levels were higher in progressors compared with non-progressors at all steps, but significantly so only in patients progressing from macroalbuminuria to ESRD compared with patients who remained macroalbuminuric (Table 2). In Kaplan–Meier estimates of progression made after dividing the patients into those with MBL levels above or below the median (Fig. 1a–c), the rate of progression from macroalbuminuria to ESRD was significantly higher among patients with high MBL ($p=0.03$, log rank test; Fig. 1c). High MBL levels remained significantly associated with progression from macroalbuminuria to ESRD after adjustment for other significant covariates (HbA_{1c}, systolic blood pressure, triacylglycerol level and total cholesterol level) in a multivariate Cox regression analysis (HR 1.65, 95% CI 1.02–2.67, $p=0.040$). The association between high MBL and progression remained the same when adding AER to the Cox analysis (HR 1.88, 95% CI 1.06–3.32, $p=0.030$). Higher hsCRP levels were seen in patients that progressed from normoalbuminuria to microalbuminuria compared with patients who remained normoalbuminuric ($p=0.018$) but not in other progressors. In a univariate analysis, the rate of progression from normoalbuminuria to microalbuminuria was significantly higher in patients with hsCRP levels above the median ($p=0.001$, log rank test; Fig. 2),

Table 1 Baseline clinical characteristics of the patients

Baseline status	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	*p* value			
				Overall	Normo- vs microalbuminuria	Micro- vs macroalbuminuria	Normo- vs macroalbuminuria
N (men/women)	1,010 (492/518)	236 (150/86)	318 (184/134)	<0.001	<0.001	0.18	0.004
Age (years)	34.0±11.5	36.7±12.1	40.0±9.5	<0.001	0.002	0.002	<0.001
Duration (years)	18.1±11.1	24.9±10.6	28.6±7.8	<0.001	<0.001	<0.001	<0.001
BMI (kg/m^2)	24.6±3.2	25.2±3.3	25.7±3.9	<0.001	0.06	0.19	<0.001
WHR, men	0.892±0.066	0.909±0.065	0.938±0.066	<0.001	0.01	<0.001	<0.001
WHR, women	0.799±0.058	0.814±0.060	0.829±0.062	<0.001	0.08	0.15	<0.001
Systolic blood pressure (mmHg)	128±16	133±17	143±20	<0.001	0.001	<0.001	<0.001
Diastolic blood pressure (mmHg)	78±9	80±10	82±10	<0.001	0.004	0.009	<0.001
HbA_{1c} (%)	8.2±1.4	8.8±1.6	9.0±1.6	<0.001	<0.001	0.08	<0.001
Triacylglycerol (mmol/l)	0.95 (0.74–1.27)	1.09 (0.79–1.50)	1.40 (1.03–2.04)	<0.001	<0.001	<0.001	<0.001
Total cholesterol (mmol/l)	4.78±0.88	4.96±0.91	5.44±1.06	<0.001	0.016	<0.001	<0.001
HDL-cholesterol (mmol/l)	1.26±0.34	1.21±0.34	1.16±0.35	<0.001	0.11	0.13	<0.001
Serum creatinine (μmol/l)	84 (75–92)	89 (81–100)	121 (96–167)	<0.001	<0.001	<0.001	<0.001
Antihypertensive treatment (%)	12	64	94	<0.001	<0.001	<0.001	<0.001
Current smoking (%)	21	29	31	0.001	0.01	0.29	0.001
24 h AER (mg/24 h)	8 (6–13)	45 (21–98)	502 (171–1278)	NA	NA	NA	NA
hsCRP (mg/l)	1.73 (1.14–2.93)	1.99 (1.24–3.52)	2.43 (1.60–4.35)	<0.001	0.008	0.001	<0.001
Men	1.51 (0.94–2.41)	1.89 (1.19–3.37)	2.40 (1.39–4.22)	<0.001	<0.001	0.012	<0.001
Women	1.96 (1.34–3.39)	2.14 (1.34–4.10)	2.51 (1.77–4.70)	<0.001	0.34	0.035	<0.001
MBL (μg/l)	1,644 (552–2,903)	1,679 (520–2,968)	2,099 (711–3519)	0.038	0.70	0.11	0.011
Men	1,791 (629–2,897)	1,997 (769–3,272)	2,016 (612–3,527)	0.30	0.27	0.84	0.18
Women	1,621 (492–2,834)	1,423 (304–2,471)	2,085 (824–3,393)	0.027	0.27	0.013	0.026

Data are mean ± SD, median (interquartile range) or percentages

Table 2 Clinical characteristics in progressors and non-progressors according to baseline albuminuria status

Baseline status	Normoalbuminuria			Microalbuminuria			Macroalbuminuria		
	Non-progressors	Progressors	*p* value	Non-progressors	Progressors	*p* value	Non-progressors	Progressors	*p* value
N (men/women)	930 (444/486)	80 (48/32)	0.035	201 (122/79)	35 (28/7)	0.029	232 (129/103)	86 (55/31)	0.18
Age (years)	34.1±11.6	32.4±10.2	0.20	37.0±12.3	35.1±11.1	0.40	40.2±9.7	39.3±9.0	0.43
Duration (years)	18.2±11.3	17.5±9.2	0.58	24.9±10.6	24.9±10.8	0.98	29.0±7.8	27.6±7.5	0.18
BMI (kg/m^2)	24.6±3.2	25.0±3.4	0.30	25.1±3.2	25.5±4.0	0.47	25.8±3.5	25.3±4.8	0.29
Systolic blood pressure (mmHg)	128±16	129±14	0.62	132±17	135±16	0.35	141±19	150±22	<0.001
Diastolic blood pressure (mmHg)	78±9	80±10	0.034	80±10	83±9	0.037	82±10	85±11	0.025
WHR male	0.888±0.067	0.923±0.057	0.001	0.904±0.064	0.934±0.067	0.033	0.935±0.058	0.947±0.082	0.241
WHR female	0.799±0.055	0.795±0.090	0.682	0.811±0.058	0.855±0.084	0.083	0.829±0.064	0.832±0.056	0.770
HbA_{1c} (%)	8.1±1.3	9.4±1.7	<0.001	8.6±1.4	9.8±2.1	<0.001	8.9±1.5	9.5±1.9	0.003
Triacylglycerol (mmol/l)	0.94 (0.73–1.27)	1.07 (0.88–1.44)	<0.001	1.02 (0.75–1.38)	1.66 (1.07–2.55)	<0.001	1.30 (0.97–1.77)	1.71 (1.22–2.94)	<0.001
Total cholesterol (mmol/l)	4.75±0.85	5.06±1.10	0.002	4.89±0.87	5.37±1.01	0.003	5.35±0.87	5.69±1.45	0.014
HDL-cholesterol (mmol/l)	1.26±0.33	1.29±0.43	0.45	1.22±0.34	1.19±0.34	0.62	1.17±0.32	1.11±0.43	0.21
Serum creatinine (μmol/l)	83 (75–92)	86 (79–92)	0.71	89 (80–98)	90 (84–107)	0.39	110 (92–137)	275 (146–366)	<0.001
Antihypertensive treatment (%)	12	11	0.95	61	83	0.01	94	97	0.31
Current smoking (%)	21	29	0.10	26	49	0.006	31	30	0.80
24 h AER (mg/24 h)	8 (6–12)	17 (10–34)	<0.001	38 (18–70)	155 (94–234)	<0.001	358 (141–857)	1,831 (727–3,191)	<0.001
hsCRP (mg/l)	1.69 (1.13–2.86)	2.10 (1.35–3.59)	0.018	1.94 (1.19–3.46)	2.42 (1.66–4.85)	0.083	2.40 (1.60–4.66)	2.65 (1.66–4.12)	0.95
Men	1.45 (0.92–2.38)	1.89 (1.18–3.38)	0.017	1.73 (1.11–3.32)	2.34 (1.67–4.69)	0.055	2.27 (1.38–4.27)	2.66 (1.41–4.17)	0.59
Women	1.89 (1.33–3.34)	2.58 (1.42–3.60)	0.13	2.13 (1.31–3.98)	2.53 (1.42–9.38)	0.46	2.52 (1.76–4.88)	2.47 (1.77–3.74)	0.58
MBL (μg/l)	1,654 (552–2,852)	1,911 (529–3,155)	0.50	1,691 (504–2,960)	1,953 (888–3,269)	0.48	1,854 (676–3,204)	2,442 (708–4,301)	0.027
Men	1,784 (631–2,856)	2,040 (572–3,339)	0.55	2,039 (547–3,181)	1,969 (937–3,415)	0.48	1,875 (614–3,308)	2,408 (525–4,340)	0.14
Women	1,618 (485–2,834)	1,734 (509–2,850)	0.82	1,427 (343–2,510)	1,210 (29–1,978)	0.52	1,809 (808–3,065)	2,633 (1017–4,288)	0.079

The *p* values refer to comparisons between progressors and non-progressors within the same albuminuria group

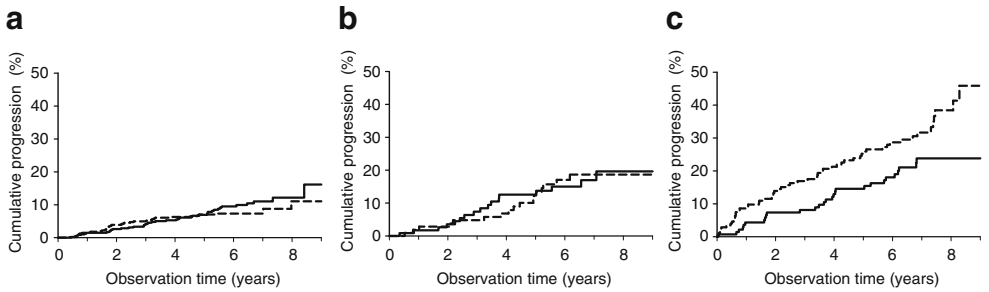

Fig. 1 Cumulative incidence of progression (**a**) from normo- to microalbuminuria (p=0.32), (**b**) from micro- to macroalbuminuria (p=0.98) and (**c**) from macroalbuminuria to end-stage renal disease (p=0.03) during follow-up according to baseline MBL levels. Solid line indicates MBL levels lower than the sex-specific median of 1,864 μg/l in men and 1,643 μg/l in women. Dashed line indicates MBL levels above the sex-specific medians. p values refer to the logrank test

but the association was only borderline significant after adjustment for other significant covariates (sex, HbA_{1c}, diastolic blood pressure, WHR and levels of triacylglycerol and total cholesterol) in a multivariate Cox-regression analysis (HR 1.56, 95% CI 0.97–2.51, p=0.068).

In patients with type 2 diabetes, combining MBL and hsCRP may provide additional prognostic information on the development of microalbuminuria [19]. In the present study, Kaplan–Meier estimates of progression made after dividing patients according to both MBL and hsCRP median levels showed a significantly higher progression rate from normoalbuminuria to microalbuminuria in patients with both high MBL and high hsCRP compared with patients with both low MBL and low hsCRP (p=0.011, log rank test; Fig. 3). However, the association was not statistically significant in a multivariate Cox regression analysis.

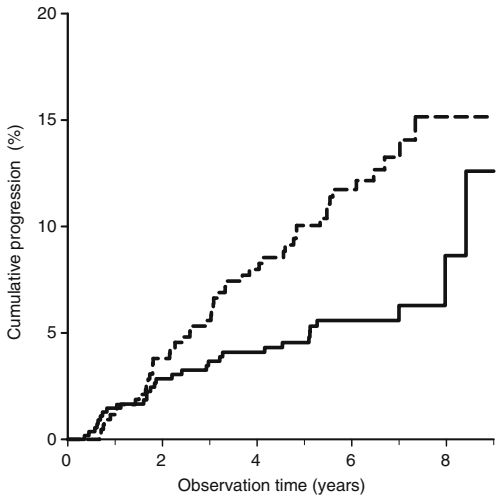

Fig. 2 Cumulative incidence of progression from normoalbuminuria to microalbuminuria during follow-up according to baseline hsCRP levels (p=0.001, logrank test). Solid line indicates hsCRP levels lower than the sex-specific median of 1.72 mg/l in men and 2.10 mg/l in women. Dashed line indicates hsCRP levels above the sex-specific median

Discussion

This large prospective multicentre study confirms previous cross-sectional findings of successively increased concentrations of MBL and hsCRP in patients with increasing severity of diabetic nephropathy. In addition, the study demonstrates that both molecules are associated with the progression of renal disease in type 1 diabetes. Our findings are in line with previous studies on the associations of both MBL and hsCRP with progression of diabetic nephropathy in type 2 diabetes [19, 22], and points to a role of complement activation and low-grade inflammation in the pathology of diabetic kidney disease.

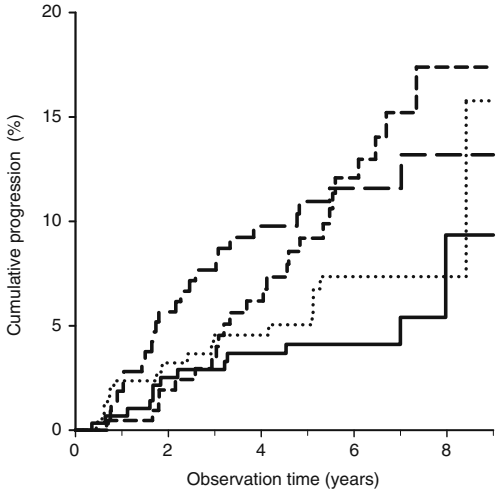

Fig. 3 Cumulative incidence of progression from normoalbuminuria to microalbuminuria during follow-up according to both baseline MBL and hsCRP levels (p=0.011, logrank test). Solid line indicates MBL and hsCRP levels below the sex-specific median of 1,864 μg/l in men and 1,643 μg/l in women for MBL and 1.72 mg/l in men and 2.10 mg/l in women for hsCRP. Line with long dashes indicates MBL levels below and hsCRP levels above the sex-specific medians. Dotted line indicates MBL levels above and hsCRP levels below the sex-specific medians. Line with short dashes indicates MBL and hsCRP levels above the sex-specific medians

The major part of inter-individual variation in MBL levels is genetically determined [7], and the fact that patients with diabetic nephropathy have higher levels than normoalbuminuric patients may thus point to a causative role of MBL. Assuming that patients born with high MBL levels are at increased risk of developing nephropathy, a higher proportion of these patients would have some degree of albuminuria already at baseline, which could explain why MBL levels were only associated with late progression from macroalbuminuria to ESRD in the present study. High-sensitivity CRP, by contrast, seems more associated with the earlier stages of progression, and may rather be a marker of progression than a causative factor. In epidemiological studies elevated hsCRP levels are consistently associated with atherosclerosis and vascular disease [23], and measurements of CRP levels have been used for therapeutic decision making in the primary prevention of cardiovascular events [24]. Polymorphisms in the CRP gene are associated with differences in circulating CRP levels [25], but it was recently demonstrated that these polymorphisms in themselves are not associated with an increased risk of ischaemic vascular disease [26]. Based on these findings it seems likely that CRP is merely a marker for the development of vascular disease, and one may speculate that the same is the case with respect to the progression of diabetic nephropathy.

Concentrations of MBL and hsCRP were not correlated in the present study or in previous studies of patients with type 1 diabetes mellitus [10, 12, 13], probably as a consequence of the strong genetic influence on MBL levels. One could therefore expect a stronger association with the progression of nephropathy if prognostic information from the two variables were combined. Among normoalbuminuric patients with type 2 diabetes, those with both high MBL and high hsCRP levels have a more than twofold greater risk of progression to microalbuminuria compared with patients with both low MBL and hsCRP [22], but in the present study the combined variable was only significantly associated with the progression of nephropathy in the univariate analysis.

Our study has some limitations. First, only samples from baseline were included, and the study does not provide information on changes over time in the concentrations of the two biomarkers. For the purpose of prognostication and possible clinical use, however, the single-sample approach seems most feasible and, with respect to MBL, the day-to-day variation [27] and changes during acute phase responses [28] are very small compared with the genetically determined person-to-person variation. Second, the prospective part of the study may have been biased by the duration of diabetes at baseline. Ideally, studies of prognostic biomarkers should include patients with newly diagnosed diabetes, but in our study the average duration was approximately 20 years, and at this stage of disease patients with the highest inherent disposition are likely to have progressed to some degree of albuminuria. This is reflected in the progression rate from normo- to microalbuminuria, which was below 10% during follow-up, compared with successively higher progression rates from the more advanced stages of nephropathy.

In summary, MBL and hsCRP levels are cross-sectionally associated with the severity of diabetic nephropathy in type 1 diabetes, and both molecules may contribute information in the assessment of risk of future progression, albeit with different strengths and at different stages of renal disease.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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